THE CHEMISTRY OF THYROID DISEASES

Publication Number 393
AMERICAN LECTURE SERIES®

A Monograph in
AMERICAN LECTURES IN LIVING CHEMISTRY

Edited by
I NEWTON KUGELMASS, M D , Ph D , Sc D
Consultant to the Departments of Health and Hospitals
New York City

The

CHEMISTRY

Of

THYROID DISEASES

By

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FOREWORD

Our Living Chemistry Series was conceived by Editor and Publisher to advance our newer knowledge of chemical medicine in the cause of clinical practice The interdependence of chemistry and medicine is so great that physicians are turning to chemistry, and chemists to medicine in order to understand the underlying basis of life processes in health and disease. Once chemical truths, proofs and convictions become sound foundations for clinical phenomena, key hybrid investigators clarify the bewildering panorama of biochemical progress for application in everyday practice, stimulation of experimental research and extension of postgraduate instruction Each of our monographs thus unravels the chemical mechanisms and clinical management of many diseases that have remained relatively static in the minds of medical men for three thousand years Our new Series is charged with the *nisus élan* of chemical wisdom, supreme in choice of international authors, optimal in standards of chemical scholarship, provocative in imagination for experimental research, comprehensive in discussions of scientific medicine, and authoritative in chemical perspectives of human disorders

Dr. Pitt-Rivers and Dr Tata of London unfold the story of thyroid hormones, their biosynthesis, circulation, physiological action, metabolism and relation to endocrine and other body systems in health and disease. The apparent simplicity of the thyroid gland a generation ago has given way to complexity in this era following the development of newer knowledge of biosynthesis of thyroid hormones, iso-

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AUTHORS' FOREWORD

The arrangement of this monograph follows in the main the usual pattern, the different manifestations of thyroid disease have been considered individually in separate chapters However in Chapter 4, this system has been abandoned and immunological aspects of all thyroid disorders have been grouped together. This has been done in order to give as clear a picture as possible of one aspect of thyroid disease which is in the early stages of investigation. Future work in this field may help to elucidate some of the etiological mysteries of hyperthyroidism and lymphadenoid goiter.

The bibliography is not comprehensive. It has been our aim to put an emphasis on the more modern literature, especially when it is too recent to have been reviewed. Many classical references have been omitted, since these may be found in all text books on the thyroid gland

topic techniques for iodinated compounds; chromatographic separation of minute amounts of thyroid and plasma components; autoradiographic localization of iodinated products, thyroxine analogues, goitrogenic agents and antithyroid compounds Recent growth of thyroid research has reached the logarithmic phase but the authors crystallize our current knowledge with critical evaluation of future trends. Several questions remain unanswered, i e, the enzyme mechanism of thyroid concentration of iodine from the blood for organic incorporation, the peripheral target of the thyroid hormones, the enzymes involved in thyroid hormone biosynthesis, and the physical chemistry of the thyroid hormones The dependence of thyroid function on the endocrine system points to a more physiologically integrated mechanism than has yet been evolved.

I NEWTON KUGELMASS, M D, PH D, SC D., *Editor*

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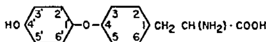
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THE CHEMISTRY OF THYROID DISEASES

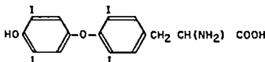
Chapter 1

NORMAL BIOCHEMICAL PROCESSES IN THE THYROID GLAND

In vertebrates, the thyroid gland possesses the property, shared by several other tissues, of concentrating iodide from the blood; however, only the thyroid has been shown unequivocally to elaborate the thyroid hormone. The early researches of Baumann, Hutchison and Oswald showed that the iodine in the thyroid was organically bound in the protein thyroglobulin The principal hormone, thyroxine, was first isolated by Kendall in 1915 and was found to contain 65% of iodine. Between the years 1926 and 1929, Harington improved the method of isolation of thyroxine from thyroid tissue, he first established by degradation and synthesis the constitution of the product obtained by catalytic deiodination of thyroxine (desiodothyroxine, thyronine), and showed it to be β-[4-4'-hydroxyphenoxyphenyl] alanine:



Later Harington established the orientation of the iodine atoms in thyroxine by similar methods and showed that they were present in the 3 5 and 3':5' positions of the thyronine molecule:



synthesis occurs in the following stages:

(1) Accumulation of iodide by the thyroid
(2) Iodination of tyrosine in thyroglobulin to monoiodotyrosine and then to diiodotyrosine.
(3) Coupling of 2 molecules of diiodotyrosine to give thyroxine.
(4) Coupling of 1 molecule each of mono- and diiodotyrosine to give triiodothyronine

The above reactions require an oxidative step before iodide can react with tyrosine, but the mechanism of the oxidation is unknown, the enzymes involved have not been isolated although Dempsey has obtained histochemical evidence of a peroxidase in the thyroid.

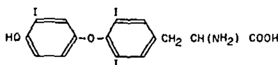
Both iodide concentration and iodine binding can be accomplished by tissues such as mammary gland and by homogenates of thyroid, spleen, submaxillary gland and seaweed However the principal compound formed in these homogenates is monoiodotyrosine, sometimes accompanied by small amounts of diiodotyrosine. The coupling reaction to give iodothyronines does not take place In order to achieve thyroid hormone biosynthesis, it appears that a definite degree of integrity of thyroid tissue is essential

The thyroid gland stores its hormones in the protein thyroglobulin This protein is soluble in water and in dilute salt solution from which it can be precipitated by ammonium sulfate or acid, it has a molecular weight of about 600,000 Thyroglobulin has a free electrophoretic mobility similar to that of γ-globulin, but when mixed with serum, it migrates with the x-globulins It is rich in arginine and there is some evidence that it is a glycoprotein

The thyroid hormones and their precursors are liberated from thyroglobulin by the thyroid protease Monoiodotyrosine and diiodotyrosine are not normally secreted by the gland though they may appear in the blood after radiation damage to the thyroid or in certain pathological conditions

3·5-Diiodotyrosine was also shown to be a major iodinated constituent of the thyroid gland (for detailed references see Harington, 1933; Pitt-Rivers and Tata, 1959). The mechanism of the biosynthesis of thyroxine from diiodotyrosine was discussed and developed by Harington in 1944

In more recent years, chromatographic techniques and the labeling of iodinated compounds with I^{131} *in vivo* have revealed other iodinated amino acids in thyroglobulin. In 1948, Fink and Fink identified 3-monoiodotyrosine in rat thyroid hydrolysates after I^{131} administration. Later Leblond, Gross and coworkers found other unknown radioactive compounds in the thyroid and blood of animals given I^{131}. One of these unknowns was also found in the blood of patients treated with therapeutic doses of I^{131}. This unknown was identified by Gross and Pitt-Rivers (1953) as 3·5·3'-triiodothyronine:



At the same time Roche and coworkers (see Gross, 1954 for detailed references) reported chromatographic identification of triiodothyronine in rat thyroid hydrolysates. Gross and Pitt-Rivers subsequently isolated triiodothyronine from fresh ox thyroid and showed that it possessed a high physiological potency This finding has been repeatedly confirmed (see Barker, 1955), and triiodothyronine is recognized as one of the thyroid hormones

Four other iodinated amino acids have been detected in the thyroid gland, namely 3·3'-diiodothyronine, 3·3' 5'-triiodothyronine and mono- and di-iodohistidine The physiological significance of these compounds is unknown, the last mentioned iodothyronines possess little or no activity when assayed in mammals.

It is now generally believed that thyroid hormone bio-

roxine (Taurog, Tong and Chaikoff, 1958). Conversely injections of anterior pituitary extract or TSH stimulate thyroid function and result in hypertrophic changes in the cells of the thyroid follicle, increased rate of thyroid hormone secretion and accelerated amphibian metamorphosis.

The normal regulation of the thyroid by the anterior pituitary has repeatedly been shown to depend upon an intact hypothalamus (see Harris, 1955; Greer, 1957; D'Angelo and Traum, 1958), although some residual pituitary function remains even after extensive hypothalamic destruction. However, the importance of intimate proximity of hypothalamus and pituitary for the proper control of the secretion of the trophic hormones including TSH has recently been demonstrated by Nikitovitch-Winer and Everett (1958); these workers found that thyroid function, which was markedly suppressed after transplantation of the pituitary to the kidney could be restored to a considerable extent by re-transplantation of the graft to the median eminence.

Thyroid function is known to vary greatly in different species For instance the rat has been described as 'hyperthyroid' while the rabbit and guinea pig are 'hypothyroid.' Recent experiments in our laboratory have shown that in the rat, mouse and hamster, 50% or more of the I^{131} reaching the thyroid becomes protein bound within sixty seconds of intravenous injection; in the rhesus monkey, five minutes are required for 50% of the I^{131} to become protein bound and in the rabbit and guinea pig this time interval is increased to thirty minutes.

Of all the exogenous factors that affect thyroid function, dietary iodine is the most important, and it is now generally recognized that extreme *iodine deficiency results in* goiter (Kelly and Snedden, 1958) Excess of iodine on the other hand depresses thyroid function. This effect has been used in the preparation of hyperthyroid patients for surgery, but the escape of the human thyroid from iodide

After proteolysis the iodotyrosines undergo deiodination (Roche, Michel, Michel and Lissitzky, 1952) by the thyroid deiodinase and the iodide thus obtained can re-enter the biosynthetic cycle.

THYROID HORMONE FORMATION IN VITRO

About twenty years ago, Ludwig and von Mutzenbecher showed that thyroxine was formed during the iodination of casein in bicarbonate buffer and later discovered that diiodotyrosine itself gave rise to small amounts of thyroxine when incubated at about pH 10. Since then, these reactions have been extensively studied in other laboratories, and artificially iodinated proteins have been used to stimulate galactopoiesis, especially in cows It has also been shown that the yield of thyroxine obtainable from diiodotyrosine *in vitro* is greatly increased if the diiodotyrosine is bound in peptide linkage. The concentration of iodine used to form iodoproteins *in vitro* is very high, as is the concentration of diiodotyrosine peptide that yields maximal amounts of thyroxine peptide. These reactions cannot therefore be said to represent thyroid hormone biosynthesis in the gland (see Pitt-Rivers and Tata, 1959).

FACTORS THAT AFFECT THYROID HORMONE BIOSYNTHESIS

In the healthy animal, the iodide concentrating mechanism of the thyroid, thyroid hormone biosynthesis and thyroid hormone secretion are all regulated by the anterior pituitary hormone thyrotrophin (TSH). The effect of hypophysectomy on thyroid function has long been recognized and results in hypothyroidism In the young animal hypophysectomy is followed by retarded growth, and metamorphosis in amphibia can be prevented by hypophysectomy as by thyroidectomy Hypophysectomy has also been shown to depress the incorporation of I^{131} into the iodotyrosines and thy-

Chapter 2

BIOCHEMICAL CHANGES IN HYPOTHYROIDISM

Hypothyroidism may arise from a number of causes. (a) an insufficiency of dietary iodine which, in its most severe form may lead to cretinism; (b) a failure of the thyroid gland to utilize efficiently the iodine that it accumulates for thyroid hormone biosynthesis; (c) the ingestion of substances, either drugs or natural foodstuffs, that inhibit thyroid function; these may all give rise to thyroid hyperplasia (non-toxic goiter) which may or may not compensate for depressed thyroid hormone production. Lastly (d), there may be a partial or complete failure of the gland to accumulate iodine, resulting in myxedema

ENDEMIC GOITER

Endemic goiter has been recognized for many centuries, and the etiology of the disease has been studied more extensively than any other aspect of thyroid dysfunction. Long before the discovery of iodine in the thyroid, an association between iodine deficiency and goiter was suggested, and more than one hundred years ago Chatin (see Harington, 1933) began a systematic investigation of the iodine content of air, water, soil and vegetable products from areas of endemic goiter in Europe. Chatin's analytical methods were unfortunately too crude to demonstrate a relationship between iodine lack and goiter, and his findings failed to convince his contemporaries; it was not until

suppression is well known, and has precluded its prolonged used in the treatment of thyrotoxicosis. Thyroid function in the rat is also depressed by iodide, but here the effect is even more transient, lasting less than 24 hours.

Other dietary factors have recently been investigated in foods whose ingestion also results in goiter (see Greer, 1950). These goitrogenic factors are found in the swede (rutabaga), groundnut, kale and other *brassicae.* They are destroyed by cooking, so that their goitrogenic action in humans is not often observed; however Clements and Wishart (1956) have shown that the feeding of large amounts of kale to cows yields milk which produces goiter in children (see chapter 2).

Environmental temperature influences thyroid function Exposure to moderate cold stimulates the thyroid, both I^{131} uptake and thyroid hormone secretion rate are increased Prolonged exposure to cold is followed by depressed thyroid function, this probably represents a reaction to stress, since other stresses such as surgical shock, burns, irradiation, starvation and injections of adrenocorticotrophic hormone or large doses of adrenocortical steroids also result in depressed thyroid function in some species Exposure to heat lowers the body's requirement of thyroid hormone and is followed by a depressed thyroidal I^{131} uptake and thyroid hormone secretion rate (see Money, 1955; Pitt-Rivers and Tata, 1959)

in these areas however, it is recognized that the iodization of salt is the best form of prophylaxis.

Goiters have been found in animals in some endemic regions. As long ago as 1907, Marine studied pathological changes in the thyroids of dogs in Cleveland, in 90% of which there was thyroid enlargement. Animal goiter has also been found in herbivores and in dogs in China, Nepal and Tasmania

Three notable studies of endemic goiter have been made with the aid of I^{131}. Stanbury *et al* (1954) investigated iodine metabolism in an endemic goiter area in Mendoza, Argentina, which lies in the foothills of the Andes. I^{131} uptake by the thyroid was greatly elevated but storage of iodine in the gland was low. There was an inverse correlation between thyroidal uptake and the urinary excretion of iodide. In 104 of the 129 subjects studied, the mean protein-bound iodine (PBI) concentration was 5 81 µg per 100 cc., and in 80% of the subjects was within the normal range of 4-8 µg per 100 cc (see Peters and Man in Werner, 1955a). Most of the goitrous subjects were clinically euthyroid. It appeared therefore that the goiter was able to compensate adequately for iodine lack, which was considered the most probable cause of the hyperplasia

Roche *et al.* (1957) have studied endemic goiter in the Venezuelan Andes; they also found high thyroidal I^{131} uptakes in their goitrous subjects In this region, where iodine deficiency was suspected but not proved, I^{131} uptakes were also very high in some people that had no thyroidal enlargement, thus the thyroid gland responded to iodine lack either by hyperplasia or by hyperactivity. Again, urinary excretion of iodide was found to be low. The PBI^{131} values fell within the range found for hyperthyroid patients, which suggested that I^{131} was more rapidly bound in the thyroid and secreted as labeled hormone than in people in non-endemic goiter areas

the beginning of the twentieth century that Marine reopened the investigation and showed that the incidence of endemic goiter in parts of North America was directly related to the low iodine content of the thyroid in man and various animals. Since that time, the relationship between iodine deficiency and endemic goiter has been the subject of many studies (see Orr and Leitch, 1929) and it is now almost universally accepted that an insufficiency of dietary iodine is the principal cause of endemic goiter.

The earliest evidence of the curative effects of iodide administration in goitrous patients came from the work of Coindet in 1820, but the deleterious effects of iodine overdosage administered by some of his followers led temporarily to the discredit of the treatment.

The first large-scale trial of the effectiveness of iodide therapy in goiter was that of Marine and coworkers more than forty years ago Goiter was prevalent among schoolgirls in Akron, Ohio, Marine showed that the growth of goiters could be prevented by the administration of 2 g of potassium iodide twice yearly. Since that time, the administration of iodine, either as potassium iodide, iodine in potassium iodide or potassium iodate (in salt) has been widely used in the prophylaxis and treatment of endemic goiter (see Matovinovic and Ramalingaswami, 1958).

Kelly and Snedden (1958) have reviewed extensively work on the prevalence and distribution of endemic goiter in a world-wide survey. they have shown that goiter has been found in all the countries investigated though in some countries (e g, Northern and Western Australia, Iceland) it is rare. In many of the endemic areas, goiter has been attributable principally to iodine deficiency, though this is not always the case. In parts of India and Burma for instance, the high calcium content of the water and the eating of considerable amounts of lime is held to be largely responsible for the high incidence of goiter. Even

Bernheim *et al*, 1956).

The use of I^{131} in the diagnosis of thyroid disease has recently been reviewed by Pochin, Jaimet and Thode, Joyet, Fauvert *et al*, Franco *et al.*, Palacios and Freedberg (see *Proc. int. Conf. on Peaceful Uses of Atomic Energy*, 10:1956) and by Veall and Vetter (1958). It is evident from these works that thyroidal I^{131} uptake and thyroid hormone metabolism are markedly depressed in hypothyroidism (see Fig. 1) Treatment of the hypothyroid patient slowly restores the

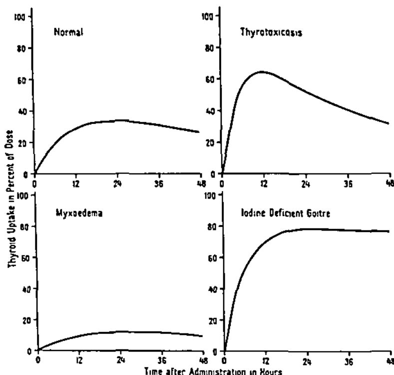


Fig 1 The uptake of a radioiodine tracer dose in the thyroid gland in different states of thyroid function (From Veall and Vetter, 1958)

rate of thyroxine degradation to normal. Urinary excretion of radioactive iodide is usually higher than normal in hy-

Lamberg and his colleagues and others (see Lamberg *et al* 1958) have studied endemic goiter on the Finnish mainland and on the Åland islands In the former study, urinary excretion of I^{131} was found to be depressed and was inversely proportional to the size of the goiter. PBI^{131} values in these patients were often subnormal or low in the normal range. In the latter study of 130 patients, similar findings were made. Further, the authors calculated that the daily formation of thyroid hormones was higher in patients with goiters than in the controls. In the group with the largest goiters, the estimated mean daily thyroid hormone production was twice that in the controls. Since the PBI values were the same, it was concluded that in these patients, the peripheral destruction of thyroid hormone and reutilization of iodide was accelerated, this was discussed in relation to a tendency towards thyrotoxicosis, which is characteristic of endemic goiter in Finland In this connexion, the high PBI^{131} levels observed by Roche *et al.* (1957) may be of interest

Endemic goiter may be associated with marked hypothyroidism which, in its most severe form, appears as goitrous cretinism and is often associated with congenital deafness The decrease in cretinism and deafness which has followed the use of iodized salt in Switzerland during the last forty years is discussed by Kelly and Snedden (1958) As we have seen, endemic goiter is not necessarily associated with hypothyroidism since most of the goitrous subjects examined in the two Andean studies were euthyroid

Although endemic goiter and cretinism may be associated, they do not always run parallel; Clements (1957) suggests that other factors besides iodine deficiency may play a part in the etiology of cretinism The prevalence of cretinism is directly related to inbreeding in certain mountainous districts, and has diminished with improvements in communication with other districts and countries (see also

parent absence in the gland was probably due to its rapid turnover.

Pitt-Rivers, Hubble and Hoather (1957) have studied 15 cases of non-toxic nodular goiter; I^{131} (about 200 µg) was given at varying time intervals before surgical thyroidectomy and both nodular and paranodular tissue was analysed chromatographically for I^{131} labeled iodo-amino acid content. Similar analyses were made on thyroid glands from 3 euthyroid subjects It was found that the formation of thyroxine in the glands of the goitrous patients was depressed or at least delayed (see Fig. 2).

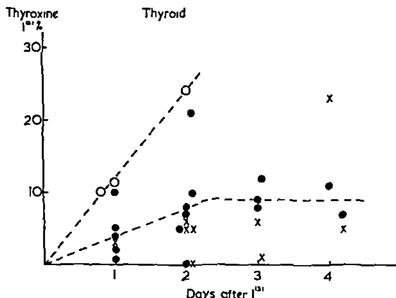


Fig 2 Thyroidal thyroxine I^{131}% in normal subjects and in patients with nodular goiter at different time intervals after administration of I^{131} Open circles represent thyroid tissue from normal subjects Solid circles represent paranodular tissue from goiters Crosses represent nodular tissue (From Pitt Rivers, Hubble and Hoather, 1957)

It was further found that in some of the goitrous tissue, there was a high monoiodotyrosine to diiodotyrosine ratio

pothyroidism.

Thyroid hyperplasia has been produced in experimental animals fed a diet low in iodine (Remington, 15 μg. I per kg.), but many of these diets are unsatisfactory and may lead to symptoms of ill-health other than iodine deficiency. These goiters disappear when the level of dietary iodine is raised to 265 μg. per kg (see Pitt-Rivers and Tata, 1959)

HYPOTHYROIDISM RESULTING FROM THYROID DYSFUNCTION

Hypothyroidism resulting from a failure of the thyroid gland to utilize iodine has been the subject of a number of studies in recent years; from the findings described below, Stanbury and Querido (1956) have postulated a classification involving inborn errors of metabolism as the cause of certain types of cretinism These are. (1) an inability to combine thyroidal iodine in organic linkage, (2) a failure to convert iodotyrosines to thyroid hormones, and (3) an absence of iodotyrosine deiodinase in the thyroid and the peripheral tissues

In 1950, Stanbury and Hedge showed that in 4 siblings three of whom were goitrous cretins, I^{131} was rapidly taken up by the thyroid gland; however the whole of it could be discharged by thiocyanate, indicating that none had been converted to organic iodine. Similar findings have been made in a few other patients (see Trotter, in Pitt-Rivers and Tata, 1959) McGirr, Hutchison and Clement (1959a) also describe patients in whom organic binding of I^{131} appears to be slowed down or impaired.

In a single goitrous cretin, Stanbury, Ohela and Pitt-Rivers (1955) found large amounts of labeled iodotyrosines in the thyroid gland after I^{131} though thyroxine could not be detected; it was however found in the blood. Its ap-

labeled monoiodotyrosine when given orally. In four patients most of the radioactivity appeared in the urine unchanged or as the conjugate, 0-2 hours after the dose, and represented 6-20% of the dose administered Two to six hours after the dose the urinary radioactivity was still mostly in the form of monoiodotyrosine and its conjugate

Fletcher *et al.* (1958) have shown that human subjects with normal thyroid function can deiodinate adequately very large amounts (up to 1 g) of I^{131} labeled diiodotyrosine given orally or up to 180 mg. given by intravenous injection, however, some "flooding" of the deiodinating mechanism was demonstrated between 0-1 hour after the dose by the appearance of considerable amounts of I^{131} labeled diiodotyro-in the urine In the second hour period after the dose, most of the urinary iodine was present as iodide except when carrier doses of diiodotyrosine exceeded 100 mg, monoiodotyrosine was completely deiodinated within ne hour of intravenous administration, even when given in a 150 mg dose. It appears therefore that Stanbury's and McGirr's patients lack both the thyroidal and peripheral deiodinase systems, or only possess them in very reduced amounts, since they are unable to deiodinate even the relatively small amounts of monoiodotyrosine and diiodotyrosine that are endogenously produced in the thyroid

It has been found (Tata, 1959, Tata and Wolff, 1959) that the thyroidal deiodinase and at least one of the peripheral deiodinases are different enzymes, with different cofactor requirements and different substrate specificities It is not possible to state from the evidence at present available, whether the deficiency of these two enzymes results from the same genetic defect.

Some aspects of goiter associated with congenital deafness have been studied by Fraser, Morgans and Trotter (1960). In thirteen families that they investigated, there were twenty-eight cases in which both goiter and deafness

$\frac{MIT}{DIT}$). In these glands, triiodothyronine was never found, although it has been found in considerable amounts in the thyroid of one patient with nodular goiter (Pitt-Rivers, unpublished). These patients do not probably fall into Stanbury and Querido's categories, but have been included here since they demonstrate either a faulty or delayed mechanism for the conversion of diiodotyrosine to thyroxine or high turnover of thyroxine.

McGirr, Clement, Currie and Kennedy (1959) have followed the metabolism of I^{131} in a case of goiter accompanied by malignant changes Thyroidal uptake of I^{131} was rapid and the serum was found to contain monoiodotyrosine, thyroxine and perhaps a trace of triiodothyronine twenty-four hours after the dose. The urine contained iodotyrosines plus unidentified compounds The failure of certain portions of the gland to utilise iodine effectively was demonstrated and in one, the $\frac{MIT}{DIT}$ was as high as 14.4:1.

Stanbury and colleagues (see Stanbury and Querido, 1956) have shown that in three goitrous patients, two of whom were cretins, the peripheral metabolism of the iodotyrosines was abnormal As we have seen (Chapter 1), these compounds do not normally appear in the circulation, since they are deiodinated in the thyroid gland In these patients, both iodotyrosines were found in the serum and urine after administration of I^{131}. Further, injection of I^{131}-labeled monoiodotyrosine and diiodotyrosine resulted in their appearance in the urine Slices from one of these glands were not able to deiodinate labeled iodotyrosines *in vitro*, although such a deiodination can readily be demonstrated with normal thyroid tissue

Gardner *et al* (1959) have studied six goitrous cretins and have shown that they could be divided into all three of Stanbury and Querido's groups

McGirr, Hutchison and Clement (1959b) have also found that some goitrous cretins are unable to deiodinate

Thiocyanate and the thiocarbamide drugs owe their antithyroid activities to different mechanisms: Thiocyanate prevents the concentration of iodide by the thyroid; the thiocarbamides do not affect iodide concentration but prevent its incorporation into organic compounds. The action of thiocyanate can be prevented by the administration of iodide; only thyroxine or compounds with thyroxine-like activity can reverse or prevent the action of the antithyroid drugs of the thiocarbamide group

Certain anions have been shown to act like thiocyanate in inhibiting thyroidal iodide accumulation. These include perchlorate, periodate and nitrate, of which perchlorate is the most powerful; it has been successfully used in the treatment of hyperthyroidism The mode of action of this group of antithyroid agents is quite unknown.

The drugs of both the thiourea and thiocyanate groups depend for goitrogenicity on a functioning pituitary gland, after hypophysectomy, no thyroid hyperplasia results. This can be explained as follows since thyroid hormone synthesis is blocked by these drugs, their administration will eventually lead to a depletion of thyroid hormone in the body, the brake on the pituitary will be removed, and there will be an increased secretion of TSH. This will stimulate thyroid cell growth and will result in goiter.

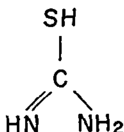
The mechanism of action of the thiocarbamide group of drugs is not fully understood, but it is thought that they act by inhibiting the enzymes involved in the iodination of tyrosine and the iodotyrosine coupling reactions. This hypothesis is supported by the observations that compounds that inhibit cytochrome-oxidase inhibit iodine incorporation into thyroid slices; that thiourea inhibits organic binding of iodine in milk, and that many antithyroid drugs prevent organic binding of iodine in casein under the influence of partially purified xanthine oxidase Peroxidase is inhibited by the antithyroid anilines, sulfonamides and resorcinol (see

occurred. In all these patients, I^{131} binding in the thyroid was retarded, as demonstrated by the perchlorate discharge test 1 hour after I^{131} administration. In the unaffected members of the families, there was little, if any change of thyroidal I^{131} after perchlorate. Chromatographic analyses of two of the goiters demonstrated an impaired ability to synthesize thyroid hormone. In all these patients, neither goiter nor deafness occurred alone It is of interest to speculate on the mechanism of control of thyroid and *auditory function.*

ACTION OF ANTITHYROID COMPOUNDS

Drugs

The first record of drug goitrogenicity was the observation of Barker in 1936 that prolonged treatment of hypertension with thiocyanate might result in goiter Several years later, this was followed by the discovery by MacKenzie and MacKenzie (1943) and Astwood and coworkers (1943) that sulfaguanidine and thiourea were goitrogenic in certain laboratory animals Since that time, a vast amount of work has been done in a search for drugs to control hyperthyroidism *This search has been made principally among* the derivatives of thiourea, the thiouracils, aminothiazoles and mercaptoimidazoles, all of which contain the grouping:



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Certain anions have been shown to act like thiocyanate in inhibiting thyroidal iodide accumulation These include perchlorate, periodate and nitrate, of which perchlorate is the most powerful, it has been successfully used in the treatment of hyperthyroidism. The mode of action of this group of antithyroid agents is quite unknown.

The drugs of both the thiourea and thiocyanate groups depend for goitrogenicity on a functioning pituitary gland, after hypophysectomy, no thyroid hyperplasia results. This can be explained as follows· since thyroid hormone synthesis is blocked by these drugs, their administration will eventually lead to a depletion of thyroid hormone in the body; the brake on the pituitary will be removed, and there will be an increased secretion of TSH. This will stimulate thyroid cell growth and will result in goiter

The mechanism of action of the thiocarbamide group of drugs is not fully understood, but it is thought that they act by inhibiting the enzymes involved in the iodination of tyrosine and the iodotyrosine coupling reactions This hypothesis is supported by the observations that compounds that inhibit cytochrome-oxidase inhibit iodine incorporation into thyroid slices; that thiourea inhibits organic binding of iodine in milk, and that many antithyroid drugs prevent organic binding of iodine in casein under the influence of partially purified xanthine oxidase Peroxidase is inhibited by the antithyroid anilines, sulfonamides and resorcinol (see

VanderLaan and Storrie, 1955; Astwood, 1955). Peroxidase activity in rat thyroid homogenates in the presence of enzymically generated hydrogen peroxide has been demonstrated by Alexander (1959). This activity is inhibited by thiocyanate, azide, thiouracil, p-aminobenzoic acid and 3-amino-1:2:4-triazole, but not by perchlorate

Dietary Goitrogens

Probably the oldest known antithyroid compound is calcium This, as we have seen, has been held responsible for endemic goiter in India, Burma and in countries where *the water has a high calcium content.* Taylor *(1954)* has shown that rats fed on a low iodine diet develop thyroid hyperplasia, which can be enhanced by the addition of up to 2% of calcium carbonate in the diet. Taylor found that calcium carbonate was still goitrogenic when the rats were given supplementary iodide up to 2 μg per day, which was thought to be the rat's normal requirement. Uptake of I^{131} by the thyroids of the calcium fed rats was not affected, but much of it could be discharged by thiocyanate and was not therefore organically bound. The mechanism of the goitrogenic action of calcium is not known.

Certain vegetable foodstuffs have been shown to contain substances that interfere with thyroid function In 1928 Chesney and coworkers found that rabbits fed on an exclusive diet of cabbage developed enormous goiters, which were largely reversible with iodide; the goitrogens therefore belonged predominantly to the thiocyanate group. A number of *brassicae* (cabbage, brussels sprouts, mustard, rape) and their seeds contain goitrogens whose action cannot be reversed by iodide; their activity can however be abolished by boiling Astwood, Greer and Ettlinger (1949) have isolated the antithyroid compound goitrin of the swede (rutabaga) and have shown it to be L-5-vinyl-2-thiooxazolidone Its precursor, progoitrin, is a glucoside (Greer, 1956)

Clements and Wishart (1956) in Tasmania found that cows fed on the *brassica* chou-moellier produced milk that was goitrogenic in calves and school-children that drank the milk; these goiters were not reversible by iodide. Wright (1958) showed that a goat fed on kale produced a goitrogen in its milk of the thiocyanate type; administration of the milk to rabbits pretreated with propyl thiouracil and I^{131} caused a partial discharge of the I^{131} from their thyroids; this goitrogen was probably thiocyanate itself. Wright suggests that some of the *brassicae* contain both types of goitrogens and will therefore inhibit both accumulation and organic binding of iodine in the thyroid.

Other foodstuffs contain goitrogenic substances (see Greer, 1950; *Nutrition Reviews,* 16: 19, 1958); whether these play an important part in the etiology of endemic goiter remains to be shown

MYXEDEMA

In 1874 Gull published the first description of adult myxedema, a condition that he considered had many features in common with cases of sporadic cretinism described three years previously by Fagge Ord later invented the name myxedema or mucous edema, because of the swelling of the subcutaneous tissues and the jelly-like substance found in them in this disease Ord further showed that the thyroid gland of one of his myxedematous patients examined post mortem had undergone marked atrophic changes, the colloid in the follicles had disappeared, and the epithelial tissue was replaced by fibrous tissue Since then various degrees of wasting of the thyroid have been demonstrated in myxedema (see Harington, 1933)

Myxedema can be divided into two types primary myxedema, which is due to a functional failure of the thyroid gland itself, and secondary or pituitary myxedema which is due to pituitary failure, as a result of the latter, TSH is

no longer secreted and the thyroid fails to get its normal stimulus.

The cause of primary myxedema is unknown; it might result from general degeneration of thyroid tissue, from a failure of the enzymes involved in iodide collection and thyroid hormone biosynthesis in the gland, or because circulating *TSH* was no longer able to stimulate its targets For a number of reasons, it has been suggested by Skillern, Goudie and their colleagues (see Trotter, in Pitt-Rivers and Tata, 1959) that primary myxedema and lymphadenoid goiter may have a similar etiology. If this is so, autoimmune

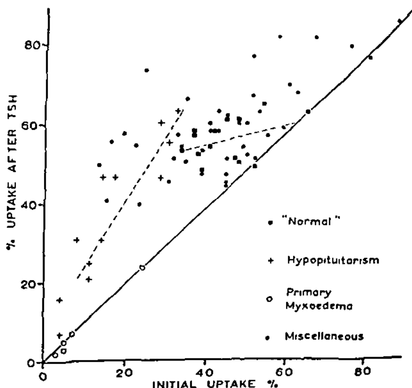


Fig 3 Effect of TSH on thyroidal uptake (% of I^{131}) in various thyroid states. Interrupted lines are regression lines through the hypopituitary (on left) and "normal groups" (From Fletcher and Besford, 1958)

reactions may play some part in the progress of myxedema (see Chapter 4).

Many laboratory tests do not differentiate between primary and secondary myxedema For instance, the range of BMR values found in the two conditions are similar, as are the ranges of thyroidal I^{131} uptakes and PBI values (see Lerman, in Werner, 1955a) Tests that reflect a general failure of pituitary function will of course give different results in primary and secondary myxedema, but probably the most critical test is the response to TSH. In primary myxedema there is none whereas in pituitary myxedema, *administration of TSH is followed by a rise in the BMR, thyroidal* I^{131} uptake and PBI in the blood

The response of the hypothyroid patient to TSH has been used by several workers to differentiate between the two types of myxedema Fletcher and Besford (1957) have shown that the thyroidal I^{131} uptake in patients with hypopituitarism is statistically greater after TSH administration (see Fig 3) In eight untreated patients, the uptake rose after TSH ("Thyrotropar" Armour) from 1 65 to 2 75 times the initial uptake, with a mean of 2.1 ± 0.33 As found by others, no patient with primary myxedema showed any change in thyroidal I^{131} uptake after TSH (see also Skillern and Evans, 1957)

Chapter 3

BIOCHEMICAL CHANGES IN HYPERTHYROIDISM

The two principal manifestations of hyperthyroidism are Graves' disease (toxic diffuse goiter, exophthalmic goiter) and Plummer's disease (toxic nodular goiter). As will be seen, most of the biochemical studies in the literature have been performed on patients with Graves' disease, but no striking differences have been found when the two types of hyperthyroidism have been compared

ETIOLOGY

The etiology of hyperthyroidism is unknown It has been suggested that it may arise from some stimulation in the brain In support of this hypothesis, Harris and Woods (1957) have shown that electrical stimulation of the hypothalamus can result in changes in thyroidal activity in rabbits, but only if they are partially or totally adrenalectomized In the intact animal, stimulation of the tuber cinereum results in inhibition of thyroidal activity Changes in thyroid activity were demonstrated in the increased or decreased rates of secretion of preformed I^{131}-labeled hormone from the animals' glands The authors suggest that the inhibition of secretion rate in the intact animals during hypothalmic stimulation results from the secretion of some substance from the adrenal glands that either inhibits thyrotrophin (TSH) secretion from the pituitary or exerts a direct effect on the thyroid, this substance is probably of adrenocortical

origin, since it has been shown that stimulation of the hypothalamus results in an increased secretion of adreno-corticotrophic hormone. Harris and Woods discuss these observations in relation to the suggestion that hyperthyroidism may be caused by emotional stress or fright and to the facts that the adrenal glands are sometimes found to have diminished function in patients with Graves' disease and that the incidence of thyrotoxicosis is raised in patients with Addison's disease. In normal man, the response to stress is an increased secretion of ACTH. If for some reason this does not occur, then the thyroid gland may get out of control since the braking action of the adrenocortical hormones cannot be exerted

Yamada and Greer (1959) have shown that injection of L-thyroxine in to the "thyrotrophin area" of the rat's hypothalamus produces inhibition of TSH secretion, with a latent period of action of six to nine hours Inhibition was immediate when thyroxine was injected directly into the anterior pituitary The effect lasted about twenty hours after either locus of administration The authors suggest that thyroid hormone control of TSH secretion may be effected by two mechanisms, a rapidly acting one which acts on the anterior pituitary itself and a slower one acting through the central nervous system

Another suggestion for the cause of Graves' disease is an increased activity of the anterior pituitary gland and a consequent increase in circulating TSH In favour of this theory are the repeated findings that injection of TSH into animals results in manifestations of hyperthyroidism (see Werner, 1955a). In 1945, Albert showed that injection of anterior pituitary extracts produces exophthalmos in the Atlantic minnow, *Fundulus heteroclitus Linn* Dobyns and Steelman later showed that the thyroid stimulating activity and exophthalmos producing activity of certain anterior pituitary extracts were not always parallel Dobyns and Wilson

(1954) have found that when the sera of certain patients with progressive exophthalmos are injected into the Atlantic minnow, exophthalmos may result. There was evidence that the response in the fish was directly proportional to the severity of the patients' disease. The authors did not know whether the substance in the serum was the same as that in the pituitary. The former was slow acting, whereas the latter acted quite quickly, furthermore, the addition of the pituitary exophthalmic producing substance to serum resulted in a more rapid response than that obtained with the patients' sera

Adams and Purves (see Adams, 1958) have recently tested pituitary extracts and sera from animals and humans for TSH activity, by measuring their effect in raising the plasma I^{131} level in thyroxine-treated guinea pigs The normal response to TSH was a more elevated level of I^{131} in the animals' plasma three hours after injection than it was sixteen hours after injection The injection of sera from some thyrotoxic patients elicited an abnormal response in that the I^{131} plasma level in the guinea pigs was higher sixteen hours after TSH injection than three hours after injection An abnormal response was also obtained with the serum of one patient who was euthyroid after thyroidectomy Sera from normal subjects elicited no response while that from a case of congenital hypothyroidism gave rise to a normal TSH response The authors postulate the existence of an abnormal form of TSH in thyrotoxicosis which may explain the etiology of exophthalmos and may give rise to the hyperthyroidism of thyrotoxicosis It might be the same substance as the "exophthalmic producing substance" of Dobyns and Wilson McKenzie (1958) has demonstrated a delayed response in mice to sera from 9 of 11 thyrotoxic patients, the sera of 3 non-toxic subjects also elicited a delayed response

Another argument in favour of TSH as the cause of hyperthyroidism comes from the work of Sonenberg, Money,

Berman, Brener and Rawson (1957). These authors have found that injection of acetylated TSH antagonises endogenous TSH in animals. It also inhibited TSH activity in hyperthyroid humans, as evidenced by a fall in protein bound iodine and I^{131} uptake by the thyroid.

The failure of many workers to find raised levels of circulating TSH or indeed any TSH at all in hyperthyroid patients forms the main objection to the pituitary origin of Graves' disease. However in 1943 Rawson, Graham and Riddell found that thyroid tissue removed at operation from patients with Graves' disease could inactivate twice as much TSH as could thyroid tissue from euthyroid subjects (with parathyroid adenomas) This might explain the absence of high circulating levels of TSH in hyperthyroidism. Nevertheless, other factors, discussed by Werner (1955a) and Trotter (in Pitt-Rivers and Tata, 1959) cast doubts on the pituitary origin of Graves' disease. Furthermore, Gurling et al. (1959) observed severe thyrotoxicosis in two women, following hypophysectomy for breast cancer.

Thirdly, thyrotoxicosis may arise as a result of a disturbance in the thyroid gland itself It is well known that the collection of iodide by the thyroid, its incorporation into thyroglobulin and the rate of secretion of thyroid hormone are all greatly accelerated in hyperthyroidism. This however may not be all, Plummer suggested many years ago that the thyroid hormone produced by the hyperfunctioning gland may be qualitatively different from that produced by the normal gland. These considerations will be discussed later.

Hereditary and epidemiological factors have also been considered in the causation of hyperthyroidism Neither of these comes within the scope of this book

THYROIDAL IODINE METABOLISM IN HYPERTHYROIDISM

Iodide

Biochemical studies in hyperthyroidism are of necessity rarer than those in hypothyroidism because of the frequent severity of the disease and the urgent need for treatment. Most of the findings have therefore been obtained during the course of diagnostic tests

The accelerated turnover of iodine in the hyperthyroid patient has been recognized for many years, but quantitative aspects were not easily investigated until the radioactive isotopes of iodine, in particular I^{131}, became available The uptake of I^{131} by the thyroid in various pathological conditions has been the subject of innumerable papers in the medical literature These consist of determinations of thyroid clearance rates, neck-thigh ratios, accumulation gradients and thyroidal uptake determinations at various time intervals after administration of I^{131} and urinary excretion tests (see Werner, 1955a, Veall and Vetter, 1958) In all these tests, there is considerable overlap in the findings for normal and hyperthyroid patients and by themselves they are not completely diagnostic; they are however useful in confirming other laboratory tests such as the determination of the basal metabolic rate (BMR), blood cholesterol, and circulating hormonal iodine

Rall (1956) has summarized some of the results of radioactive iodine tests in various thyroid diseases, it is apparent from the data quoted by him how greatly increased the thyroidal clearance rate of iodide from the circulation may be The normal value for euthyroid subjects ranges from 5 to 40 cc per minute In untreated hyperthyroid patients the clearance rate ranges from 40 to 1,500 cc per minute

Schumacher, Keating and Albert (1958) have shown that thyroid slices from patients with exophthalmic goiter, when

incubated in a medium containing I^{131}, cleared ten times as much I^{131} from the medium as did slices from normal thyroid glands, while the thyroid: medium (T/M) ratios were six times greater than normal. The significance of these findings is not clear, since protein binding in the hyperthyroid tissue was only one third that in the normal tissue, and thiocyanate caused marked discharge of I^{131} from unblocked slices as well as those blocked with methimazole. It may be that in these *in vitro* experiments the iodide-concentrating mechanism is unimpaired while iodine incorporation may be affected by tissue damage, the nature of the medium or other unknown factors Roche, Michel, Deltour and Michel (1952) have shown that there are no qualitative differences in the thyroglobulins extracted from glands from normal subjects and hyperthyroid patients.

The rate of formation of thyroid hormone was first only estimated indirectly (see Riggs, 1952; Berson, 1956) but in 1949 Stanley described a method for the direct estimation of thyroid hormone formation in man. This consisted in the administration of I^{131} followed by estimation of thyroidal I^{131} uptake; simultaneously, the radioactivity and I^{127} were estimated in the urine If the radioactivity in the serum was also determined, then serum iodide became:

$$\text{serum } I^{127} = \frac{\text{serum } I^{131} \times \text{urine } I^{127}}{\text{urine } I^{131}}$$

It was found by this method that the thyroids of fourteen euthyroid subjects accumulated an average of 10 ug I^{127} per hour, in thirteen thyrotoxic patients the value rose to an average of 120 μg per hour

Thyroid hormone secretion rate in various thyroid states has been the subject of many studies The earlier ones have been reviewed by Riggs (1952) Among the more recent studies are those of Berson and Yalow (1954), Ingbar and Freinkel (1955) and Solomon (1956). Berson and Yalow, using the I^{131} turnover method, compared the secretion rates

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first used in the preoperative treatment of thyrotoxicosis by Plummer in 1923, and still is in use today.

Stanley (1949) showed that there were quantitative differences in the effects of iodide on the thyroid glands of thyrotoxic patients and euthyroid subjects: a serum concentration of 5μg. I^{127} per 100 cc. or less inhibited iodine binding in the former group whereas 6-12 μg I^{127} per 100 cc. was necessary to inhibit iodide binding in the latter Stanley suggested that iodide inhibited thyroid hormone synthesis by interfering with the tyrosine-iodinating mechanism. Wolff and Chaikoff (1948) have shown that in the rat iodide interferes only transiently with thyroid function, although I^{131} uptake is inhibited immediately after a dose of I^{127}, the gland escapes within a few hours of the inhibitory effect. These findings have been confirmed by Galton and Pitt-Rivers (1959), the latter authors have further found that at the time that I^{131} uptake is most depressed there is a high monoiodotyrosine diiodotyrosine ratio in the gland The inhibitory action of iodide on I^{131} uptake by the thyroid gland in hyperthyroid patients has also been studied by VanderLaan (1957), Feinberg, Hoffman and Owen (1959) and others with similar conclusions: The thyrotoxic gland is much more sensitive to iodide than is the normal gland.

The very rapid secretion of thyroid hormone in hyperthyroidism has long been recognized and has been described as a "thyroid diarrhea." Iodide administration again has a much more potent effect on thyroid hormone secretion rate in the patient with Graves' disease than in the normal subject This has recently been investigated by Ansell and Miller (1952), Greer and DeGroot (1956), Solomon (1956), Goldsmith, Herbert and Lutsch (1958) and Feinberg *et al* (1959). All these workers found that small amounts of iodide markedly slowed down or completely inhibited the appearance of I^{131}-labeled hormone in the

of thyroid hormone in nine hyperthyroid patients and in euthyroid subjects (including treated hyperthyroids). In the thyrotoxic subjects, 420-925 μg hormonal iodine was secreted daily by the gland; in the euthyroid group the secretion rate was 77-161 μg per day Ingbar and Freinkel's findings were similar the thyroidal secretion rate in their hyperthyroid group was 530-1101 μg of hormonal iodine per day.

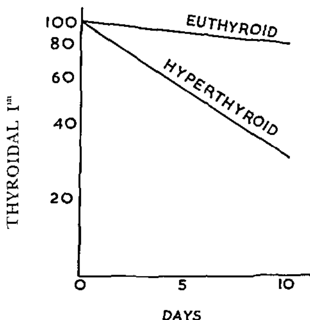


Fig 4 Release of I^{131} from the thyroid in euthyroid and hyperthyroid subjects

Fig 4 shows the greatly accelerated secretion rate of I^{131} labeled thyroid hormone from the gland in hyperthyroidism (from data in Solomon, 1956)

FACTORS AFFECTING THYROID FUNCTION IN HYPERTHYROIDISM

Probably one of the most striking effects on thyroid function in hyperthyroidism is that of iodide itself This was

of thyroid hormone secretion after 75-100 μg triiodothyronine. Only slight suppression of thyroidal secretion was obtained in the thyrotoxic subjects with much higher doses of the hormone. Again, 6 mg of thyroxine caused a decrease in the thyroidal secretion rate in a normal subject which outlasted the effect on the BMR and protein bound iodine (PBI); the same dose of thyroxine had no effect on secretion rate in a patient with diffuse toxic goiter. It is worth noting that in one hyperthyroid patient, 75 μg of triiodothyronine per day suppressed I^{131} uptake by the thyroid while thyroidal secretion rate was unaffected by 600 μg. of triiodothyronine per day. The authors suggest that hyperthyroidism results from autonomous activity of either the thyroid or pituitary gland and is not merely due to a decrease in the hypothalamic-pituitary control mechanism resulting from suppression by the circulating thyroid hormones

THE CIRCULATING THYROID HORMONES IN HYPERTHYROIDISM

The discovery of more sensitive methods for the analysis of iodine has greatly increased the demand for the estimation of protein-bound iodine (PBI) or butanol-extractable iodine (BEI) of serum as an aid to the diagnosis of thyroid disorders, since they represent the levels of circulating thyroid hormone. Determinations of PBI values on normal, hypothyroid and hyperthyroid individuals indicate the normal range varies between 4 and 8 μg per 100 cc with a mean of about 5 μg In hypothyroid patients the PBI value is generally below 2 μg per 100 cc, in hyperthyroidism the range of values is large, overlapping the upper end of the normal range but rising as high as 40 μg per 100 cc (Peters and Man, in Werner, 1955a) This reflects the rapid turnover of thyroidal iodine in the hyperthyroid patient but gives no direct indication of the nature of the hormone secreted

As we have seen (Chapter 1) the principal circulating

blood, while similar doses of iodide had no effect on the secretion rate in non-toxic subjects It was also found that moderate doses of TSH were able to neutralize the iodide effect, and returned the thyroidal secretion rate to normal in spite of continued iodide administration.

Goldsmith *et al* (1958) do not subscribe to the theory that iodide acts primarily on the thyrotoxic gland itself; they suggest that it acts by "neutralizing" thyrotrophin after it has been secreted by the anterior pituitary. They further conclude from their findings that the hyperthyroidism of Graves' disease is due to the maintenance of a peripheral concentration of thyrotrophin equal to that which would be produced by daily injection of not more than 6 mg TSH.

Thyroid Hormones

It has been shown by Greer (1951), Morgans and Trotter (1952) and Starr and Liebhold-Schueck (1953) that the administration of desiccated thyroid, thyroxine or triiodothyronine will suppress I^{131} uptake in normal subjects and in treated hyperthyroid patients (see Werner, 1955b for above references) Werner has confirmed these findings. 75-150 µg. of triiodothyronine daily given by mouth produced a marked suppression of the thyroidal twenty-four hour uptake of I^{131} in forty-eight euthyroid patients, but had little or no effect in patients with diffuse goiter, in ten patients with the eyesigns of Graves' disease without hyperthyroidism, I^{131} uptake was not depressed by triiodothyronine, however treated toxic goiter patients responded normally These observations form the basis of a simple, rapid test for hyperthyroidism

Johnson, Solomon and Greer (1959) studied the effects of thyroxine and triiodothyronine administration on thyroid hormone secretion rates in twelve normal subjects and fifteen patients with diffuse toxic goiter. All the normal subjects showed a rapid and almost complete suppression

one-dimensional solvent systems Triiodothyronine was only detected in three sera from the euthyroid group, in none was there any iodotyrosine Ten of the sera from the hyperthyroid patients showed the presence of triiodothyronine, but in no case was it present in greater amounts than the thyroxine, contrary to the findings of Benua, Dobyns and Nimmer When triiodothyronine was detected, it varied in amounts from 1/20 to 1/8 of the amount of thyroxine These authors agree with Dobyns and his group that triiodothyronine does not appear in the blood as a result of radiation damage since it could be detected as early as six hours after the administration of radioiodine These studies did not reveal the existence of any abnormal thyroid hormone.

In recent years, the transport of the thyroid hormone has been studied by many workers and has been attributed to protein (s) (thyroxine binding protein, TBP) migrating in an electrophoretic field between the x-globulins TBP was first shown to be qualitatively similar in hyper- and euthyroidism Later it was found that it did not vary quantitatively in these thyroid states. In this connexion it is of interest to observe that in pregnancy, the TBP level is raised. This probably represents a physiological adjustment to the raised TBP levels found in pregnancy, and prevents the subject becoming hypothyroid by preventing a fall of thyroxine levels in the tissues In hyperthyroidism the increased blood hormone levels are not bound by increased amounts of TBP, so that more thyroid hormone gets into the peripheral tissues than in the normal state (Robbins and Rall, 1957)

Farran, Lea, Goolden and Abbatt (1959) have found that the plasmas of some thyrotoxic patients to whom tracer doses of I^{131} were administered contained significant amounts of mono- and di-iodotyrosine, these amino acids were found in forty-three sera out of ninety two examined The patients

hormone is thyroxine, but it is now generally believed to be accompanied by small amounts of triiodothyronine. When triiodothyronine was discovered as a normal constituent of the thyroid gland and was found to be more active than thyroxine in certain biological tests and more rapid in its action in others, interest was reawakened in Plummer's hypothesis of an abnormal thyroid hormone in thyrotoxicosis

Benua and Dobyns (1955) made chromatographic studies of the sera of patients with Graves' disease treated with I^{131}, the largest detected amount of triiodothyronine was 19%. The authors pointed out that the amount of triiodothyronine was related to the degree of thyrotoxicosis and not to radiation effects, since it appeared in the blood before radiation damage would be expected Monoiodotyrosine (up to 11% of the total radioactivity) was found in the sera of fourteen patients and diiodotyrosine was found in the sera of six patients (up to 5%); all these subjects had Graves' disease

In another study, Benua, Dobyns and Ninmer (1955) examined the sera of thirty-nine patients with Graves' disease and seven euthyroid patients with heart disease treated with I^{131} In the thyrotoxic patients, the sera of twenty-two out of the thirty-nine contained triiodothyronine; in one of two sera from patients with toxic nodular goiter, triiodothyronine was present; it was only detected in two sera from the seven euthyroid subjects. It was also observed that in the first hours after radioiodine administration, triiodothyronine was found in greater amounts than thyroxine, and in a few cases, in the absence of thyroxine

Arons and Hydovitz (1959) have recently made extensive chromatographic studies of I^{131}-labeled thyroid hormones in twenty-six euthyroid subjects given 1.5-20 mc I^{131} and twenty-eight patients with toxic diffuse goiters given 1-10 mc. I^{131} Serum samples were analysed from 1 to 144 hours after the radioiodine dose. In all cases, thyroxine was the major constituent of the serum, as identified in three

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In recent years, the transport of the thyroid hormone has been studied by many workers and has been attributed to protein (s) (thyroxine binding protein, TBP) migrating in an electrophoretic field between the x-globulins TBP was first shown to be qualitatively similar in hyper- and euthyroidism Later it was found that it did not vary quantitatively in these thyroid states. In this connexion it is of interest to observe that in pregnancy, the TBP level is raised This probably represents a physiological adjustment to the raised TBP levels found in pregnancy, and prevents the subject becoming hypothyroid by preventing a fall of thyroxine levels in the tissues In hyperthyroidism the increased blood hormone levels are not bound by increased amounts of TBP, so that more thyroid hormone gets into the peripheral tissues than in the normal state (Robbins and Rall, 1957)

Farran, Lea, Goolden and Abbatt (1959) have found that the plasmas of some thyrotoxic patients to whom tracer doses of I^{131} were administered contained significant amounts of mono- and di-iodotyrosine, these amino acids were found in forty-three sera out of ninety-two examined The patients

with iodotyrosines in their plasmas had greater mean gland weights than those without; they were also more resistant to radioiodine therapy. The authors consider it unlikely that the iodotyrosines appeared as a result of radiation damage since the patients only received 100-200 μc. I^{131} though this is not excluded. The authors give no indication of the amounts of iodotyrosines which would be circulating in these patients. Since the iodotyrosines have very short biological half-lives, their presence in the blood is surprising, and as Fletcher (1957) has suggested, probably results from peripheral enzymic breakdown of thyroglobulin

THYROID HORMONE METABOLISM IN HYPERTHYROIDISM

Work on iodine metabolism in the thyrotoxic subject compared to the normal has been summarized by Riggs (1952) and others As we have seen, the uptake of iodine and secretion of thyroid hormone is accelerated in hyperthyroidism, it also appears that the rate of disappearance of thyroid hormone from the blood occurs at an accelerated rate Ingbar and Freinkel (1955) showed that exogenous I^{131}-labeled thyroxine disappeared at a faster rate in thyrotoxic subjects than in normal humans, in the latter group a mean value of 53 6 ± 16.5 μg thyroxine was degraded per day, and this rose to 359 ± 150 μg per day in untreated hyperthyroid patients In the same year, Sterling and Chodos made similar studies and also found that exogenous thyroxine degradation was accelerated in thyrotoxicosis. The biological half-life ($T_{½}$) of thyroxine in normal subjects averaged 6 2 days and fell to an average value of 2.7 days in the thyrotoxic patients

In a preliminary report in 1955, Ingbar and Freinkel described a more rapid degration rate of exogenous I^{131}-labeled thyroxine in treated hyperthyroid patients who had become euthyroid than in normal subjects This report was

later (1958) elaborated. It showed that in fifteen hyperthyroid patients, thyroxine $T_{1/2}$ averaged 4 8 ± 0 08 days; this was significantly lower than the normal value for $T_{1/2}$ of 6 8 ± 0.5 days In seven hyperthyroid patients studied after thyroidectomy (7 months to 10 years) thyroxine $T_{1/2}$ was 6 0 ± 0.9. Ingbar and Freinkel's patients were, from their data, clinically euthyroid and laboratory data gave normal values for BMR etc.

This finding has not been confirmed. Sterling (1958) showed that the degradation rate of exogenous labeled thyroxine became normal or subnormal in nineteen out of twenty hyperthyroid patients after therapy Changes were seen as soon as one month after subtotal thyroidectomy, in two cases the degradation rate became as low as that found in myxedema after treatment Friis (1958) has published results in agreement with Ingbar and Freinkel and Sterling and Chodos as regards the shortened half-life of labeled thyroxine in hyperthyroid patients In seven normal subjects $T_{1/2}$ for thyroxine was 7.0 - 9.1 days, in three hyperthyroid patients it was 3 3 - 5.7 days However, after thyroidectomy, when the patients had become euthyroid, the $T_{1/2}$ value in five patients rose to 7.0 - 10.0 days There is at present no explanation for the discrepancies between these groups of workers

Chapter 4

IMMUNOLOGICAL ASPECTS OF THYROID DISORDERS

As the title indicates, we shall not emphasize any one type of thyroid disease in this chapter. However, much of the recent knowledge gathered on the immunological or immunochemical aspects of normal and pathological thyroid function is based on the initial studies in patients with Hashimoto's disease. Hashimoto's disease, first described in 1912, is also called *Struma Lymphomatosa, lymphocytic thyroiditis*, lymphoid or lymphadenoid goiter or lymphomatous hypertrophic thyroiditis. Clinically the disease is manifested by a goiter which is medium or large, very frequently of recent origin, and of a diffuse nature. The disease is more common in women than in men The goiter is generally accompanied by hypothyroidism, although hyperthyroidism may occur during the early stages Pathologically, lymphocytic infiltration is characteristic of the goiter and it is not uncommon to find it associated with thyroid carcinoma and non-toxic nodular goiter. For details of clinical and pathological manifestations and classification of Hashimoto's disease, the reader is referred to Werner (1955a) and Luxton (1957) Interest in the immunological findings in subjects with Hashimoto's disease is principally due to the fact that these studies have prompted the investigator to suggest certain mechanisms involved in its etiology For the sake of convenience, the problems of human thyroid disease and experimental studies in animals have been considered separately

OBSERVATIONS ON HUMAN SUBJECTS

As early as 1911, Papazolu demonstrated a positive complement fixation reaction between serum from hyperthyroid subjects and thyrotoxic thyroid gland extract whereas a normal subject's serum failed to react. Many years were to pass before immunological techniques were seriously applied to the study of thyroid disease. Our knowledge of the processes of autoimmunity in thyroid diseases has been collected during the last five or six years. The approach of clinical observation preceding experimental work began with the demonstration by Fromm, Lascano, Bur and Escalente in 1953 and later by Luxton and Cooke (1956) and Skillern's group (1956) that a large amount of γ-globulin, associated with abnormal floculation tests, was found circulating in the blood of patients with Hashimoto's disease Investigation of the nature of this excess γ-globulin by Roitt, Doniach and their colleagues (1956) showed that it actually represented a large amount of precipitating autoantibody against thyroglobulin These authors used an extract of normal thyroid gland and serum from patients with Hashimoto's thyroiditis to demonstrate precipitation At about the same time, Witebsky and his colleagues had also postulated the presence of such autoantibodies in human chronic thyroiditis but as their original approach was experimental, we shall refer to their work later on

Roitt, Doniach and their colleagues have followed up their original observations with extensive researches on the nature of autoantibodies and the antigen itself (Doniach and Roitt, 1957; Roitt and Doniach, 1957, Roitt, Campbell and Doniach, 1958; Roitt and Doniach, 1958) By using Ouchterlony's technique of agar gel diffusion and precipitation they were able to show that serum from a Hashimoto patient reacts not only with crude thyroid extracts but with "purified" thyroglobulin, this is shown in Figure 5 Doniach

and Roitt (1957) also found that the antibody level diminished markedly in treated Hashimoto patients



Fig 5 Use of Ouchterlony's diffusion precipitation technique in agar gel to demonstrate thyroglobulin as the active antigen in extracts of human thyroid Center well serum from patient with Hashimoto's disease, well no 1 purified thyroglobulin 5 mg/ml, well no 2 saline extract of thyroid (1 10), well no 3 purified thyroglobulin (10 mg/ml), well no 4 saline extract of thyroid (1 20) (From Doniach and Roitt, 1957)

At this point it should be emphasized that so far no one has isolated pure thyroglobulin, Robbins, Wolff and Rall (1959b) have moreover demonstrated the heterogeneous nature of most thyroglobulin preparations It is therefore not surprising that Roitt, Campbell and Doniach (1958) found antibodies in the sera of some patients against two or three distinct antigens present in "purified" human thyro

globulin. These authors have also shown that these antibodies are both organ-specific and species-specific, within a narrow range When measurements were made of the molar ratio of antibody to antigen, using C^{14}-labeled thyroglobulin, the values were found to vary between 4:1 and 2:1. Amounts as high as 5.2 mg. of antibody protein per ml of serum have been found, this unusually high level of antibody concentration has made the study of immunological aspects of thyroid diseases particularly attractive to investigators interested in the study of human precipitating autoantibodies

From the more direct experimental evidence obtained in animals, it has been postulated that the destruction of the thyroid gland may result from a progressive interaction in the gland between the thyroglobulin and circulating autoantibodies Once initiated, this reaction would then become self-perpetuating because damage to the thyroid by the antibodies would cause more antigen to be released and hence increase the level of circulating antibodies. That the antigen responsible for the production of autoantibodies is localized in the thyroid has been elegantly shown by White (1957) using Coon's technique of fluorescent antibody. He showed that the antigen is present within the follicles by treating a frozen section of thyroid of a patient with Hashimoto's disease with the patient's own serum globulin previously made fluorescent. Careful examination of the morphological distribution of fluorescence revealed that the antigen of the thyroid colloid freely diffused out and came into contact with cells of the granuloma. White has suggested that the pathological picture of the thyroid in Hashimoto's disease represents in most cases the late stages of an immune cellular response to prolonged and continuous stimulation of antigen production As a matter of fact he has observed the early stages of the auto-immune process in some patients with low titers of antibody The fluorescent antibody technique has also been applied to the study

of the experimental production of chronic thyroiditis in rabbits by Witebsky's group.

Methods based on precipitation have been useful in demonstrating the circulating auto-antibody in patients with Hashimoto's disease However these methods are not sensitive enough and patients with low antibody titers could easily be missed Two highly sensitive methods are now used for detection of such antibodies, not only in patients with Hashimoto's disease but with other thyroid disorders They are the methods of tanned red cell agglutination and complement fixation; these have given varying degrees of satisfaction to different workers in the detection of the presence of auto-antibodies in cases of Hashimoto's disease One reason for this variance may well lie in the different criteria used by clinicians to establish the diagnosis of Hashimoto's thyroiditis before serological tests are carried out There is however no doubt about the extreme sensitivity of these two techniques, and serum titers up to 1 2,000,000 have been detected by the tanned red cell agglutination method

The availability of techniques more sensitive than the precipitation method has resulted in a large number of immunological investigations in patients with Hashimoto's thyroiditis and other thyroid diseases (White, 1957; Witebsky, 1957, Witebsky, Rose, Terplan, Paine and Egan, 1957, Trotter, Belyavin and Waddams, 1957, Goudie, Anderson, Gray, Clark, Murray and McNichol, 1957, Witebsky, Rose and Shulman, 1958, Roitt and Doniach, 1958, Owen and Smart, 1958, Doniach, Roitt, and Hudson, 1958; Stuart and Allan, 1958, Anderson, Goudie and Gray, 1959a, b, Cline and Selenkow, 1959, Belyavin and Trotter, 1959) While it is impossible to review the individual merits of these findings, it can be said that the inclusion of a large variety of thyroid disorders in immunological studies has radically modified the original perspective of auto-immunity and has

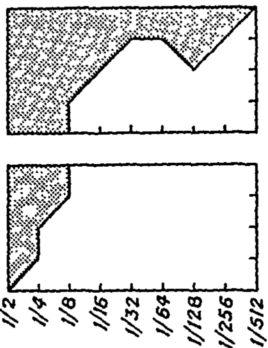
forced us to adopt a more flexible hypothesis for the etiology of Hashimoto's thyroiditis. The observation that autoantibodies reacting identically with those in Hashimoto serum are found circulating in patients with "spontaneous" or primary myxedema, probably denotes a common pathological process in the two diseases. Roitt and Doniach (1958) and Owen and Smart (1958) who carried out tests on large numbers of subjects came to the same conclusion, namely, that 80% of all the patients with primary myxedema or non-goitrous hypothyroidism had antibodies against thyroglobulin, compared to 5% in normal subjects. Roitt and Doniach applied all the three available techniques, i e., precipitin reaction, complement-fixation and hemagglutination (coating tanned human erythrocytes with human thyroglobulin), to sera from other patients and found that more than half of the thyrotoxic patients (64%) had antibodies compared with 31% in simple goiter cases. The lowest incidence of auto-immunity was detected in the group of patients with thyroid carcinoma (29% or less); in spite of this low incidence, a positive serological test in thyroid carcinoma can confuse the diagnosis although thyroid malignancy itself might arise from lymphadenoid goiter; further, the two conditions might occur together It is likely that in all thyroid diseases, positive serological reactions indicate the same auto-immune reaction occurring in differently localized forms, starting with some kind of lymphocytic infiltration. The clinical evidence of these disorders would only become apparent when the process has reached an advanced stage. Histological examination of thyroid tissue in patients with low antibody titers but without pronounced clinical signs of disease would be helpful in establishing whether the same common phenomenon occurs in a wide variety of cases

In the course of serological studies of Hashimoto's disease and other diseases of the thyroid gland, some attention

has also been devoted to the nature of antigens against which the antibodies are directed. From the early studies, it was concluded that thyroglobulin was the antigen reacting with the precipitating antibodies However with the more frequent use of the complement fixation test it has been found that thyroid extracts obtained from thyrotoxic patients were a better source of antigen than extracts from normal tissue (Trotter, Belyavin and Waddams, 1957; Roitt and Doniach, 1958; Anderson, Goudie and Gray, 1959a). Trotter *et al* (1957) were the first to suggest that an antigen quite different from thyroglobulin might be involved in the complement-fixation reaction, a suggestion which has now been amply confirmed by the work of Roitt and Doniach (1958), Anderson *et al* (1959a, b) and by Belyavin and Trotter (1959). Anderson and his colleagues have used the term of "'thyrotoxic' complement-fixation reaction" in describing this antigen, the relatively large amounts of it present in extracts of thyrotoxic glands can be estimated from Figure 6

In the course of the demonstration that the complement-fixing antibody in Hashimoto or other sera was distinct from that directed against thyroglobulin, Roitt and Doniach (1958) and Belyavin and Trotter (1959) also showed that this complement-fixing antigen was principally localized in the microsomal fraction of thyroid cells and not in the colloid Although the majority of Hashimoto patients have both thyroglobulin and complement-fixing antibodies, they may be present independently in some cases There is still much to be learnt about the nature of this non-thyroglobulin antigen, however, Anderson *et al* (1959a, b) have shown that the 'thyrotoxic' complement-fixation reaction represents an auto-immune process with a single antigen-antibody sys-

Fig 6 Complement fixation patterns obtained with 2 human Hashimoto sera (subjects Br and McG) when tested against normal and thyrotoxic gland extracts (From Belyavin and Trotter, 1959)


Serum B2
Serum McG
THYROTOXIC EXTRACT
NORMAL GLAND EXTRACT
1/5
1/10
1/20
1/40
1/80
1/1
1/2
1/4
1/8
1/16
1/2
1/4
1/8
1/16
1/32
1/64
1/128
1/256
1/512
INITIAL DILUTIONS

tem. Although the antigen is more abundant in thyrotoxic glands, it is also a constituent of normal thyroid tissue. The same authors have also shown that the sera of a small proportion of patients with Hashimoto's disease (9 out of 53) contained, in addition to the anti-thyroglobulin antibody and the 'thyrotoxic' complement-fixation antibody, another complement-fixing antibody which reacts with thyroglobulin. Thus a second type of complement-fixation reaction can occur, although Anderson *et al* doubt whether the antibodies concerned have any cytotoxic effects, they think that such effects might explain the discrepancies observed earlier when normal thyroid extracts were used instead of extracts of thyrotoxic glands The two types of complement-fixation reaction have also been described by Belyavin and Trotter (1959) who differentiate them as "antibody-sensitive" and "antigen-sensitive," the antigen in the latter case being thyroglobulin When the antigen concerned in the "antibody-sensitive" reaction (which is localized in the cellular particulate, microsomal fraction) was examined, it was found to be almost free from iodine This interesting finding accentuates the difference between this antigen and thyroglobulin and further work on its nature will certainly prove fruitful, in view of the heterogeneity of thyroidal proteins demonstrated by Robbins *et al* (1959b). Not only would it help in the better understanding of the auto-immune processes and the etiology of lymphadenoid goiter and primary myxedema, but it might also contribute to the elucidation of biochemical changes in thyrotoxicosis.

THE EXPERIMENTAL APPROACH

So far we have seen the immunological picture that accompanies the clinical manifestations of certain thyroid disorders The initial stages of the auto-immune reaction can however only be examined in experimental animals Our knowledge of the actual causation of auto-immunity

in animals and its comparison with observations on human subjects is mainly due to the work of Witebsky and his group. To these authors, serological studies in humans have been secondary to the study of an experimental condition produced in animals that is histologically and serologically similar to human thyroiditis (struma lymphomatosa). Their work has not only been of interest to thyroid biochemistry but has constituted a challenge to the accepted principles of immunology.

Until recently, immunologists considered that only foreign proteins could act as antigens; this conclusion resulted from a failure to produce auto-antibodies experimentally and was expressed by Ehrlich as "horror autotoxicus" However, despite inadequate evidence, many human diseases were ascribed to the formation of autoantibodies, classical examples being certain blood dyscrasias, also described as "autoimmune blood diseases" In 1927, Hektoen, Fox and Schulhof showed that antibodies to saline extracts of thyroid from the dog, goat, guinea pig, horse, rabbit, sheep, bear, deer, racoon and zebra were highly organ-specific but not species-specific. A similar finding was reported by Stokinger and Heidelkerger (1937) but it was only several years later that Witebsky and Rose (1956) (also Rose and Witebsky, 1956) succeeded in producing iso antibodies by sensitizing one rabbit to the thyroid extract of another rabbit They accomplished this using Freund's adjuvant technique for stimulating antibody production and the tanned-cell hemagglutination method for detection of antibodies. These studies have been followed by others and Witebsky and his colleagues conclude that these thyroid antibodies are true auto-antibodies (Witebsky *et al.*, 1957, Witebsky, 1957; Witebsky, Rose, Paine and Egan, 1957; Witebsky, Rose and Shulman, 1958, Ovary, Randall, Witebsky, Rose, Shulman and Metzgar, 1958) The two principal reasons for believing this are. a) thyroidectomized rabbits

develop circulating thyroid antibodies when injected with extracts of their own thyroid glands, and b) rabbit thyroid antibodies produced in normal rabbits bring about marked



Fig 7a

Fig 7 Histological similarity between the damage to thyroid tissue a) produced in a rabbit sensitized to thyroglobulin and b) in a human subject suffering from chronic thyroiditis (From Witebsky Rose Terplan, Paine and Egan 1957)

histological changes in the thyroid gland. In humans, additional evidence comes from the fact that thyroid extracts from different individuals give almost identical titers when tested against a serum sample from a patient with chronic thyroiditis. More recently Witebsky, Rose and Shulman (1958) have actually shown a reaction between serum and



Fig. 7b

extracts of thyroid tissue removed from the same patient, thus distinguishing between isoantibodies and autoantibodies.

The histological changes brought about in thyroid tissue as a result of sensitization to thyroid extracts is one of the main links between experimental and clinical observations. Witebsky's group has produced degenerative changes in the thyroid glands of the dog, guinea pig and rabbit. The structural damage in the rabbit thyroid was found to be roughly proportional to the autoantibody titer in the serum, as estimated by precipitation, complement-fixation and tanned-cell hemagglutination The striking histological similarity between the thyroid gland from a rabbit, three months after an intradermal injection of rabbit thyroglobulin plus Freund's adjuvant, and that of a patient with chronic thyroiditis can be seen in Figure 7
In both cases there is considerable diminution of normal colloid and dense infiltration by lymphocytes, plasma cells and eosinophils Because of this similarity between experimental thyroiditis and human thyroiditis, Witebsky and his colleagues examined serum specimens from patients with chronic thyroiditis and other thyroid diseases Their serological findings have been essentially similar to those described previously However, in some patients with histologically proven thyroiditis, they were unable to detect any circulating auto-antibodies Nevertheless, they have put forward the hypothesis that some types of chronic thyroiditis represent an autoimmunization process within the patient against his own thyroid tissue It is assumed that a slow but continuous release of thyroglobulin into the circulation will act as a constant antigenic stimulus, resembling the depot effect in animals treated with Freund's adjuvants Although under normal conditions thyroglobulin is not secreted into the blood-stream, the demonstration of a thyroglobulin-like protein circulating in the blood of patients with Hashimoto's

disease supports this assumption (Owen and McConahey, 1956; Stemmermann, 1956). This hypothesis in no way tells us how thyroglobulin would come to be released in the first place; viral infection has been thought a likely agent Furthermore, the finding of auto-antibodies in patients with other thyroid disorders and the presence of a thyrotoxic complement-fixation factor indicates that some modification of the above hypothesis is necessary

Witebsky and his colleagues have also worked on the nature of the antigen involved in the autoimmune reaction They have performed serological tests with different fractions of thyroid proteins and conclude that thyroglobulin is the main antigen. More recently they obtained five fractions from saline extract of a human thyroid from a patient with chronic thyroiditis by ammonium sulfate precipitation; they confirmed that the fraction richest in thyroglobulin (as seen by sedimentation in the ultracentrifuge) was also the most active in the agglutination inhibition test, while the "thyralbumin" fraction and the supernatant obtained after the precipitation of the thyroglobulin were the least active From what we have already learnt about the complement-fixation test with an iodine-poor microsomal thyroid fraction in thyrotoxic glands, we can only conclude that thyroglobulin is the principal but not the only antigen involved in this auto-immune reaction

Apart from the work on fluorescent antibodies (White, 1957), little is known about the localization of anti-thyroid antibodies The experiments of Anigstein and his colleagues on the distribution of antibodies are of interest in this connexion (Anigstein, Hardin and Whitney, 1956; Anigstein, Eklund and Whitney, 1957) By labeling the γ-globulin fraction of rat-thyroid antiserum with I^{131}, they were able to find that the antibody was primarily localized in the thyroid, with extensive cross-reactions in the adrenals, liver

and kidney An interesting aspect of their work is the reduction in the basal metabolic rate of rats injected with thyroid antiserum.

In conclusion, a completely new perspective for understanding thyroid diseases has arisen in the last few years The first immunological findings in a relatively rare disease now apply to most thyroid diseases and have led to a new approach to problems of thyrotoxicosis The experimental work is probably the best model study available of autoimmunization. It has resulted in many questions: Why should patients with Hashimoto's disease exhibit complement-fixing antibodies to an antigen found in a thyrotoxic gland? Is thyrotoxicosis the precursor of Hashimoto's disease? Does the resemblance between experimental damage to thyroid and degeneration seen in humans represent the end-effects of identical pathological processes? Foremost among these problems is the mechanism which initiates the leakage of antigen from the gland and culminates in a disease of which only the advanced stages are understood.

Chapter 5

CANCER OF THE THYROID

Although thyroid cancer is rare (only 6 out of 100,000 deaths in the United States were reported as due to this disease in 1952), it has been no less intensively studied than other thyroid diseases Most effort during the last ten to fifteen years has been devoted to the recognition of different histological characteristics of neoplastic thyroid tissue, and pathologists can now classify cancer of the thyroid in about six distinct groups Investigation has also been carried out on the problem of the etiology of this disease especially as regards external factors such as geographical distribution of iodine, diet and radiation. Little agreement has been reached as a result of these investigations except in one instance: X-irradiation of the thymus in children is recognized as a causal factor Very little has been done on the biochemical phenomena involved in the different histological types of this disease, but recently a characteristic biochemical defect in thyroid cancer has been established. The chief interest of this defect lies in its similarity to biochemical function in embryonic thyroid tissue (Tata, 1958).

As with the chemistry of other thyroid diseases, most of our present knowledge developed with the aid of radioactive iodine and microanalytical techniques. In 1942, Keston and his colleagues discovered that in certain subjects, carcinomatous thyroid tissue could concentrate radioactivity after administration of I^{131}. The demonstration of functional activity in neoplastic tissue has now been confirmed by many clinical investigators It should be pointed out however that

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lation. Such is also the case in thyroid cancer even when no normal tissue is present. Robbins and his colleagues (Robbins, Rall, Becker and Rawson, 1952; Robbins, 1954; Robbins, Petermann and Rall, 1954) have shown that the radiation-induced circulating thyroglobulin does not differ from the thyroglobulin present in normal human thyroid tissue. This was concluded from physical properties such as sedimentation in the ultracentrifuge, electrophoretic mobility and solubility in salt solutions. Thyroglobulin is not normally present in the blood of subjects with thyroid cancer, although recent immunological studies (Chapter 4) have revealed the presence of circulating autoantibodies to thyroglobulin in thyroid carcinoma (Doniach, Roitt and Hudson, 1958; Roitt and Doniach, 1958). However the percentage of carcinoma patients presenting a positive reaction was lower than patients with any other thyroid disorder. The significance of autoantibodies in the etiology of thyroid carcinoma is not known

The similarity between the thyroglobulin formed in thyroid carcinoma was demonstrable in both normal and abnormal tissue removed from the same individual (Easty, Slater and Stanley, 1958), these thyroglobulins showed no qualitative differences although there were three or four antigenic components in both proteins as revealed by Ouchterlony's method

The presence of circulating thyroid hormones has been observed together with thyroglobulin in the blood of cancer patients after administration of I^{131} Robbins *et al.* (1952) demonstrated thyroxine in the serum of athyreotic subjects with functioning thyroid metastases in lung, neck, liver and bone, using a combination of chromatography and PBI^{131} and butanol-extractable I^{131} estimations after both "tracer" and "therapeutic" doses of radioiodine With regard to triiodothyronine, this was first found in human plasma (Gross and Pitt-Rivers, 1952) from a patient with functioning thyroid

in most cases of thyroid cancer the abnormal tissue has lost the ability to concentrate iodide and is non-functional. From the biochemical viewpoint, thyroid cancer as a whole may therefore be divided into two categories of functional and non-functional types. The description below concerns only functional thyroid cancer. There are no data on variations in functional activity according to the different histological types of cancer tissue but it seems that functioning tissue is more frequently found in metastatic tissue and in papillary and follicular carcinoma.

Since very little is known about the normal mechanism of iodide concentration, it is difficult to say whether this mechanism differs in thyroid cancer from the normal. However, it has been possible to determine whether iodide concentration is followed by its incorporation into proteins and, if so, what is the nature of the iodoprotein thus formed

NORMAL IODOPROTEINS AND THYROID HORMONES IN THYROID CANCER

In 1944, Leiter, Seidlin, Marinelli and Baumann described a thyroidectomized patient who became hyperthyroid as a result of the activity of extensive metastatic thyroid cancer and who was successfully treated with radioiodine Many cases are now known of subjects with thyroid cancer who are clinically euthyroid in the absence of all normal tissue. This suggests that carcinomatous tissue is capable of forming thyroglobulin or some iodoprotein that can elaborate the thyroid hormones Direct evidence for the formation of thyroglobulin in thyroid cancer was first obtained during the examination of circulating radioactivity in patients treated with large doses of I^{131}. In subjects with normal thyroid tissue or in those who suffer from diseases of the thyroid other than cancer, it is known that administration of a large dose of I^{131} will be followed by the appearance of large amounts of thyroglobulin in the circu-

AN ABNORMAL IODOPROTEIN IN THYROID CANCER: COMPOUND X

During their studies on the nature of radioiodinated substances in the blood of patients with thyroid cancer treated with I^{131}, Robbins, Rall and Rawson (1953) reported the discovery of a labeled product quite different from thyroglobulin or any of its constituent iodo-amino acids. The study of this substance was pursued in twenty-three subjects with functional carcinoma, to whom thirteen tracer and thirty-seven therapeutic doses had been administered (Robbins, Rall and Rawson, 1955). This substance, named Compound X, was shown to possess the following major characteristics. 1) Unlike thyroxine and triiodothyronine, it was not butanol-soluble and was chromatographically immobile in acidic and alkaline butanol, 2) it was precipitated with serum proteins, 3) it differed from thyroglobulin from the same patients in its iodinated amino acid composition, its solubility in salt solutions, its electrophoretic mobility and sedimentation in the ultracentrifuge. With respect to the last two criteria Compound X behaved like *serum albumin*. It is therefore quite simple to differentiate Compound X from thyroglobulin, thyroxine and triiodothyronine using a combination of paper chromatography and electrophoresis.

Compound X has only been found in the blood of patients with thyroid cancer (to the extent of 60% of the cases reported by Robbins *et al.*, 1955), and unlike thyroglobulin, it is not released into the blood as a result of radiation damage. Robbins *et al.* (1955) demonstrated its presence in the circulation of patients with thyroid carcinoma after administration of tracer doses of I^{131}, thus indicating that this abnormal material is normally circulating in this disease.

Tata, Rall and Rawson (1956) undertook a more detailed study by selecting a few cases of highly functional cancer in whom previous studies had shown that Compound

metastases Later, Robbins, Rall and Rawson (1955) detected I^{131}-labeled triiodothyronine in the serum of nine out of fifteen radioiodine-treated patients with highly functioning carcinomata; some of these subjects were athyreotic. Triiodothyronine was present in amounts usually detected in euthyroid patients (about 1-7% of serum organic I^{131}) while thyroxine was detected more frequently in larger amounts Since then other reports have appeared of the detection of both thyroid hormones in thyroid cancer but caution should be exercised in the quantitative interpretation of these data after administration of therapeutic doses of I^{131}, especially in cases in which all the constituent iodinated amino acids of thyroglobulin are found in blood and urine (Horst and Heuwieser, 1957, Dorta and Béraud, 1959). The peripheral hydrolysis of thyroglobulin released after I^{131} irradiation of the thyroid is especially marked two-three days after I^{131} administration, this could explain the large amounts of labeled hormones found in blood and the artefactual presence of all the iodinated amino acids in the urine Hence, results obtained with "tracer" or low doses of I^{131} or analyses carried out at early time-intervals after the dose have more significance.

The hormones in the blood of thyroid carcinoma subjects appear to be transported in the normal manner. This has been shown by zone electrophoresis of serum from subjects to whom radioiodine was given (Horst and Rösler, 1953, Robbins, Rall and Rawson, 1955), no difference could be detected in the characteristics of the thyroxine-binding protein (TBP) of carcinomatous and normal subjects. From these considerations it can be seen that athyreotic subjects (either from birth or due to a previous thyroidectomy) with thyroid cancer may display a euthyroid status and even on rare occasions may develop hyperthyroidism

of Compound X free from serum albumin. The increased thermal stability of serum albumin in the presence of fatty acid anions (Teresi and Luck, 1952) was the basis of the second test, and measurement of radioactivity in precipitate and supernatant fractions after heat coagulation in the presence and absence of 0 02 M-sodium caprylate clearly separated Compound X and serum albumin

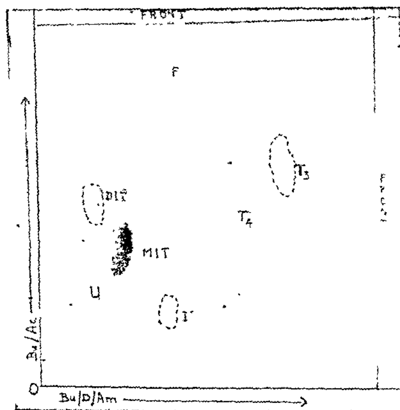


Fig 8 Radioautogram of two dimensional chromatographs of hydrolysate of I¹³¹ labeled compound X using butanol acetic acid (Bu Ac) and butanol dioxane ammonia (Bu/D/Am) as developing solvent systems The areas surrounded by the dotted lines represent the positions of substances absent from the hydrolysate (positions known from the carriers added) (From Tata, Rall and Rawson 1956)

X circulated in relatively large amounts. The minute amount of total iodine in blood (40-100 μg. per litre) necessitated the study of the nature of Compound X after labeling with I^{131} Blood was collected at various time intervals after the administration of 50-250 mc. of I^{131} and Compound X was obtained by isolating serum albumin. This was done by one of the following procedures: a) separation by zone electrophoresis on a starch block, b) fractional salting out with potassium phosphate and c) Cohn fractionation with ethanol. During the last procedure, it was accidentally found that zinc ions in the presence of ethanol would remove any thyroxine or triiodothyronine bound to TBP or albumin Thus it was possible to obtain Compound X free from thyroglobulin and thyroid hormones in the blood Physical examination of the isolated material by electrophoretic studies, solubility tests and sedimentation in the ultracentrifuge, revealed a close resemblance to serum albumin (Robbins, *et al*, 1955, Tata *et al.*, 1956)

At this stage, attempts were made to separate albumin from Compound X in order to decide whether the abnormal material was iodinated albumin itself, an iodinated substance bound unusually firmly to albumin or an iodinated protein distinct from albumin The latter was considered the most likely possibility from tests based on two highly specific properties of human serum albumin (Tata *et al.*, 1956) For both tests albumin labeled with varying amounts of I^{131} and albumin to which radioactive thyroxine was bound were used for comparison In the first method, which was immunological, rabbit anti-human serum albumin antiserum was used to distinguish Compound X from albumin. The difference was best seen by measuring the radioactivity in precipitate and supernatant fractions in a precipitin curve obtained from albumin mixed with Compound X and radioiodinated albumin. The immunological method also offered the possibility of isolating small amounts

therapeutic dose of I^{131}. On the other hand, the level of Compound X had reached a maximum within twelve to eighteen hours after the dose and was maintained at that level for a long period of time. Since this abnormal iodinated substance is normally present in the blood of carcinoma patients (in the absence of radiation damage), the above data are interpreted to mean that it is turned over rapidly into the blood, although nothing is known about the turnover time at the site where it is formed. When the same samples of Compound X and serum thyroglobulin

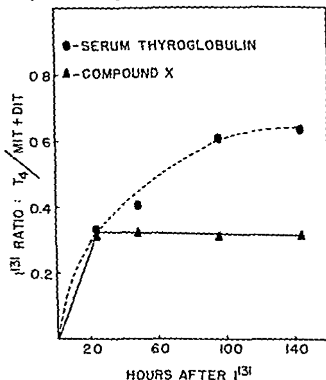


Fig 9 Comparison of the rate of incorporation of I^{131} into the iodothyronine and iodotyrosine fractions of compound X and serum thyroglobulin isolated simultaneously from the blood of the same subject which functioning thyroid carcinoma T_4 = thyroxine like component, MIT = 3 monoiodotyrosine; DIT = 3 5-diiodotyrosine (From Tata, 1958)

The protein nature of Compound X is revealed by its susceptibility to proteolytic enzymes. When tryptic or alkaline hydrolysis was carried out, 3-iodotyrosine was found to be the major radioactive iodinated constituent (Robbins *et al., 1955; Tata et al., 1956*). Small amounts of 3:5-diiodotyrosine, thyroxine and one or more unidentified I^{131}-labeled compounds were also detected by chromatographic analysis. In some cases, as shown in Figure 8, 3-iodotyrosine was found in the virtual absence of diiodotyrosine.

In this case, *it is difficult to explain the presence of what* appears to be thyroxine in the absence of diiodotyrosine, since the latter is the precursor of thyroxine (Harington, 1944) This could be explained if what appears to be thyroxine were in fact 3:3'-diiodothyronine, since it could be formed by the condensation of two molecules of monoiodotyrosine, further, the chromatographic properties of the two substances are very similar in the solvents used. A few attempts by Tata *et al* (1956) to identify 3:3'-diiodothyronine in hydrolysate of Compound X have however failed The chromatographic identification of thyroxine in hydrolysates of iodoproteins with a high monoiodotyrosine: diiodotyrosine ratio has also been reported in cases of nodular goiter (Pitt-Rivers *et al.*, 1957, see Chapter 2).

This qualitative difference between Compound X and thyroglobulin was further apparent in kinetic studies on the rate of incorporation of radioiodine into the two proteins and their release into the circulation The fractions of I^{131} administered to a subject with highly functional carcinoma which appeared in the circulation as Compound X and thyroglobulin as a function of time are shown in *Figure 9.*

As is well known, the discharge of thyroglobulin into the blood due to radiation damage is a slow process and it is emphasized in this case by the fact that the amount of labeled thyroglobulin was still increasing a week after the



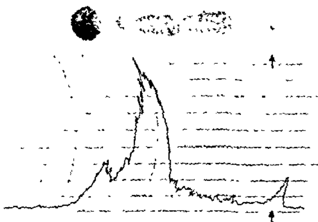


Fig 10a

Fig 10 Differences between the physico chemical properties of soluble iodoproteins extracted from normal human thyroid and from follicular adenoma a) Zone electrophoresis on paper of unfractionated I^{131} labeled iodoproteins dissolved in normal human serum The stained paper strips are mounted above the corresponding records of distribution of radioactivity b) Sedimentation of the unfractionated saline extracts in the ultracentrifuge (59 900 rpm, 0 4% solution in 0 15 M NaCl) The values above and below the schlieren patterns give the sedimentation coefficients of the corresponding boundaries (From Robbins Wolff and Rall 1959b)

shown in Figure 9 were hydrolyzed and the distribution of radioactivity in the iodinated amino-acids compared, a further interesting difference was seen In thyroglobulin isolated from the subject at increasing time intervals after the dose, the ratio of I^{131} in the iodothyronine fraction to the iodotyrosine fraction increased with time. Such a pattern is typical of thyroglobulin synthesis in normal thyroid gland With the Compound X fraction the iodotyrosine: iodothyronine ratio reached a maximum in a short time and was the same for all successive time intervals No convincing explanation has been found to account for this peculiarity; it would be necessary to determine specific radioactivity values in the hydrolyzed samples and also to make sure that what appears to be thyroxine in Compound X is at least a product of iodotyrosine condensation We have already seen (Chapter 2) that Pitt-Rivers, *et al* (1957) have described a similar difference in the hydrolysis products of normal and nontoxic nodular goiter thyroid tissues.

It is not known to what extent the differences in kinetics of incorporation of radioiodine into Compound X and thyroglobulin are related to the more rapid overall iodine turnover and decreased iodine pool in subjects with thyroid carcinoma described by various authors (Pochin, Cunningham and Hilton, 1954, Reynolds, Corrigan and Hayden, 1953). Corrigan and his colleagues have suggested that a diagnosis of thyroid cancer can be made on the basis of kinetics of iodine metabolism, this suggestion has been criticized by Sonenberg and Rall (1956) in whose opinion the secretion of Compound X could explain the kinetics of iodine metabolism in thyroid cancer while rapid iodine turnover and a small pool are also characteristic of other thyroid disorders

Very little is known about the site of formation of Compound X but it is safe to assume that it is elaborated by the functional carcinomatous tissue Stanley (1956) frac-

was done by comparing the I^{131}-labeled iodoproteins from normal tissue and the adenoma by electrophoresis and sedimentation in the ultracentrifuge (Figure 10)
Many of the criteria used for characterizing abnormal iodoproteins were identical to those used earlier for Compound X in the blood of subjects with functional thyroid cancer (Robbins, Rall and Rawson, 1955; Tata, Rall and Rawson, 1956) and a striking similarity may be observed in the properties of Compound X and "S-1 iodoprotein." Hence it is likely that "iodoprotein S-1" is itself secreted into the blood or is the precursor of Compound X

Another approach to this problem has been made in the recent work of Robbins and his collaborators (Robbins, Wolff and Rall, 1959a; Wolff, Robbins and Rall, 1959) on the iodoproteins in thyroid tissue and blood of animals with transplanted functional thyroid tumours. The iodoproteins in tumor tissue were labeled *in vivo* and *in vitro* with I^{131}. Two iodoproteins besides thyroglobulin were again detected; one of these, the "thyroid S-1 iodoprotein," possessed properties similar to the "S-1 iodoprotein" in human thyroid adenomata described previously. In tumor-bearing rats, an iodoprotein called "serum S-1 iodoprotein" was also found and it was concluded that in human thyroid carcinoma the abnormal circulating iodoprotein is secreted by the functional carcinomatous tissue. It is of interest to note that Wolff, Robbins and Rall (1959) have shown that not all transplantable rat thyroid tumors will elaborate iodoproteins since, by studying another line of tumors, they have shown that iodide trapping did not lead to any organification

tionated the iodinated proteins of normal and carcinomatous human thyroid tissues from the same subject after administration of I^{131}; he also examined metastases from lung, mediastinum and thyroid and found that the solubilities of iodinated proteins extracted from abnormal tissue differed from those of normal thyroid. The higher solubility in ammonium sulfate of the abnormal material suggested a similarity to Compound X Although Stanley did not characterize Compound X as such by electrophoresis or chromatography, he showed that 85% of the iodotyrosine fraction of carcinoma iodoprotein consisted of monoiodotyrosine Recently Robbins, Wolff and Rall (1959b) have fractionated iodoproteins in normal and abnormal human thyroid tissue by a combination of centrifugation, "salting out" and electrophoresis, they found two other types of iodoproteins besides thyroglobulin, one of these, termed "S-1 iodoprotein," was found in particularly large amounts in a follicular thyroid adenoma, while normal tissue contained much less, this

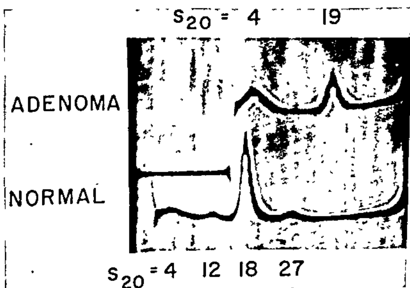


Fig 10b

to Robbins *et al.*, 1955, 1959a,b; Tata *et al.*, 1956). The difference in composition of iodinated amino acids and the fractionation studies on normal and tumor tissues rule out the possibility that Compound X is a fragment of thyroglobulin.

The high monoiodotyrosine content of Compound X is of interest and suggests that the iodination of tyrosine to diiodotyrosine via monoiodotyrosine is a process controlled by different factors at each step. We have already seen in Chapter 2 that the iodoprotein in some nodular goitrous tissue may be similarly rich in monoiodotyrosine. The similarity between the two iodoproteins in the two conditions is not only confined to the high monoiodotyrosine content. The dynamics of incorporation of I^{131} in the iodotyrosine fractions and what appear to be the iodothyronine fractions of the abnormal iodoproteins are identical to those found in nodular tissue from goiters (see Figure 2) when compared with the process occurring in the normal thyroglobulin of the same individual. Such kinetic studies have not been extended to other diseased thyroid tissues but there are other conditions, summarized below, when the iodination of tyrosine in proteins does not proceed beyond the monoiodotyrosine stage: 1) Experimental disruption of the cellular integrity of thyroid tissue has been shown to cause a block in the conversion of monoiodotyrosine to diiodotyrosine in the iodoprotein synthesized Thus Taurog, Potter and Chaikoff (1955) found that whereas thyroid slices incubated with I^{131} elaborated a normal labeled thyroglobulin, homogenization of the same tissue resulted in an iodoprotein containing virtually nothing but labeled monoiodotyrosine In humans, Costa and Cottino (1957) have found that severe surgical trauma of the thyroid gland resulted in the formation of thyroglobulin rich in monoiodotyrosine. 2) In the chick, rabbit and rat fetal thyroid it has been shown (Trunnell and Wade, 1955, Waterman and Gorbman,

SIGNIFICANCE OF THE CIRCULATING IODOPROTEIN (COMPOUND X) IN FUNCTIONAL THYROID CANCER

The above considerations show that the functional adaptation which is observed in carcinoma of endocrine tissues in general is also characteristic of thyroid carcinoma. This is particularly true regarding the elaboration and secretion of *thyroxine and triiodothyronine* However, the presence of an iodoprotein normally present in the blood of patients with functional thyroid cancer constitutes the unusual biochemical feature of abnormal thyroid growth In no other pathological condition of the thyroid, apart from traumatic or radiation damage, is an iodoprotein so frequently detected in the blood It is now known that it is not the formation of an iodoprotein such as Compound X that is the unique feature of tumor tissue but its release into the circulation For example Robbins *et al* (1959a, b) have shown that the "S-1 iodoprotein" in man and rat is found both in normal thyroid and tumor tissue, but it is only in animals with functional thyroid cancer that such an iodoprotein or Compound X is present in the blood

Any explanation of this defect in biochemical terms is still speculative The breakdown in the mechanism that prevents leakage of iodoproteins from thyroid tissue may be common for thyroid cancer and lymphadenoid goiter in which a leakage of thyroglobulin occurs (see Chapter 4) It could be due to a defective action of proteolytic enzymes or, at a morphological level, to a failure in the retention of these proteins It is also possible that thyroglobulin and Compound X are formed in different cells, which would explain the appearance of Compound X in the blood in the absence of thyroglobulin In comparing the leakage of thyroglobulin and Compound X, one should consider that the former protein has a molecular weight of about 600,000 or ten times that of the circulating iodoprotein ($S_{20,w}$ for Compound X and S-1 iodoprotein = 4 2 - 4.3 according

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1956) that a stage exists, soon after I^{131} accumulation has begun, when monoiodotyrosine is the only organic iodine compound in thyroid tissue hydrolysates; this is followed by iodination to diiodotyrosine and eventually to the formation of iodothyronines. Thyroid tissue is developed from the primitive gut and it has been shown that some other tissues that originate in the primitive gut can concentrate iodine and may in some cases incorporate it into an iodoprotein Mammary tissue is one of these, but the iodoprotein it secretes into milk only contains monoiodotyrosine and an unknown compound chromatographically similar to thyroxine (Taurog, Potter, Tong and Chaikoff, 1956; Potter and Chaikoff, 1956, Brown-Grant and Galton, 1958) The similarity between the iodinated constituents of milk iodoprotein (Brown-Grant and Galton, 1958) and Compound X (Tata *et al*, 1956, see Figure 3) is striking. It is also of interest that some primitive invertebrates which have acquired the ability to concentrate iodine have scleroproteins rich in monoiodotyrosine (see Roche and Michel, 1951).

We may conclude firstly that the iodoprotein secreted by functional thyroid carcinoma tissue resembles in iodinated amino-acid composition that formed by normal thyroid tissue whose cellular integrity has been destroyed. Secondly, the failure to incorporate iodine beyond the monoiodotyrosine stage in Compound X indicates that the metabolism of iodine in the carcinoma tissue has stopped at some early stage resembling that of normal embryonic thyroid tissue In a broad evolutionary biochemical sense, the chemistry of abnormal iodoprotein in thyroid cancer has given an opportunity to demonstrate a possible functional regression in neoplastic endocrine tissue.

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